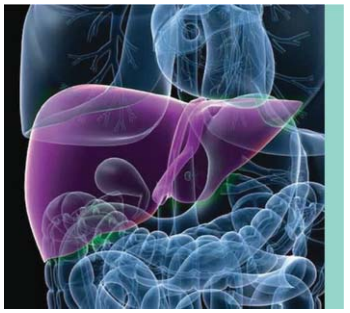

Standard Liver Tests

George Kasarala, M.D., and Hans L. Tillmann, M.D.*,†,‡*

The liver is the largest organ in the body and arguably the most important organ for protein production and detoxification, both of which are facilitated by a myriad of enzymes. Both the detection of enzymes released from liver cells and proteins produced by the liver and released into the blood can be used to analyze liver health.

Standard liver tests (Tables 1 and 2) that assess injury to the liver include alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatases (APs). The excretory function of the liver can be estimated by bilirubin and the metabolic function of the liver by clotting tests and albumin.

Tests that describe injury of the liver such as aminotransferases and AP have historically been mislabeled liver injury tests (LIT). In contrast, standard tests such as albumin, bilirubin, and prothrombin time are useful in evaluating liver function.

The pattern of elevation of the different enzymes can be used to discriminate hepatocellular from cholestatic or mixed injury; AST and ALT are more elevated in patients with hepatocellular injury, whereas AP and γ-glutamyl transpeptidase (gGT) are more elevated in cholestatic injury.

DISCRIMINATION OF NORMAL VERSUS HEALTHY VALUES

Normal values for laboratory results are defined as those found in 95% of a population. Thus, 2.5% of a population will be above and below the normal values, respectively. But being outside the normal does not immediately reflect illness; that is, a bilirubin level below normal has no clinical consequences. Contrarily, being within the normal value does not necessarily reflect a healthy state. In that regard it has been suggested to use an upper limit of 19 and 30 U/L for ALT for women and men, respectively,[1] to reflect healthy values. This also fits the observed increased mortality in individuals with ALT values that are normal but above the healthy range.[2] Thus, liver transaminases likely will be described as healthy ($\leq$19 U/L for women and $\leq$30 U/L for men) and

Abbreviations: AIH, autoimmune hepatitis; ALT, alanine aminotransferase; AMA, antimitochondrial antibodies; AP, alkaline phosphatase; AST, aspartate aminotransferase; DILI, drug-induced liver injury; ERCP, endoscopic retrograde cholangiopancreatography; gGT, γ-glutamyl transpeptidase; LIT, liver injury test; MELD, Model for End-Stage Liver Disease; MRCP, magnetic resonance cholangiopancreatography; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cholangitis; ULN, upper limit of normal.

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TABLE 1. STANDARD LIVER TESTS, THEIR SOURCES OF ORIGIN, AND ABNORMALITIES

Parameter	Origin	Associated Disease
AST	Liver, skeletal muscle, cardiac muscle, red blood cells, brain, pancreas, lungs	Hepatocellular injury of any cause, myopathies, myocardial infarct, hemolysis
ALT	Liver, kidneys, skeletal muscle	Hepatocellular injury of any cause, myopathies
AP	Liver, bone, placenta, kidneys, intestines	Cholestatic liver disease; sarcoidosis; pregnancy; lymphoma; bone, kidney, and intestinal diseases
γ-Glutamyl transferase	Biliary epithelial cells, kidneys, pancreas, prostate	Biliary or pancreatic disease, myocardial infarct, renal diseases, chronic lung disease, diabetes
Conjugated bilirubin	Hemolysis, insufficient excretion from the liver	Severe liver injury from any cause Rotor syndrome, Dubin-Johnson syndrome
Unconjugated bilirubin	Hemolysis	Hemolysis, Gilbert syndrome, Crigler-Najjar syndrome
Albumin	Produced in hepatocytes	Low in nephrotic syndrome, malnutrition, protein-losing enteropathy
Prothrombin time	Clotting factors produced in hepatocytes	Prolonged in liver disease, vitamin K deficiency, fat malabsorption, pancreatic insufficiency

normal values (i.e., <65 U/L). The normal values will depend on the specific laboratory population, thus limiting standardization.

For some assessments such as drug safety, the times upper limit of normal (ULN) is established to define safety margins. Substituting the healthy range values for normal range value will therefore need to be carefully addressed in the future.

Mild abnormalities in liver-related tests may warrant repeat testing before a more extensive workup is initiated. Abnormal liver chemistries may occur in 1% to 4% of the asymptomatic population.[3,21]

MARKERS OF HEPATOCELLULAR INJURY

Transaminases are involved in transferring the amino groups of aspartate and alanine to ketoglutaric acid.

TABLE 2. ELEVATION OF LIVER CHEMISTRIES WITH LIVER DISEASES

Test	Hepatocellular	Cholestatic	Half-life ($t_{1/2}$)
AST	+++	N/+	17 hours
ALT	+++	N/+	47 hours
AP	Normal/mild	++++	7 days
gGT	++/+++	++++	26 days (for abstinence)
Total bilirubin	N/++	N/+++	Depends on albumin binding
Albumin	++ (chronic)	N	20 days
Prothrombin time	++	N	

Although ALT is more liver specific, elevated ALT levels are also reported in myopathies (Table 3).[4,5]

Hepatocyte injury results in altered cell membrane permeability causing the excessive leakage of transaminases. Periportal hepatocytes (zone 1) have relatively more ALT, whereas the hepatocytes near the central vein (zone 3) have more AST (Fig. 1). Thus, causes of hepatic inflammation that are predominantly involving zone 1 such as viral and autoimmune hepatitis result in predominantly ALT elevation. In contrast, ischemic or toxic insults are more likely to involve zone 3, causing a predominance of AST elevation. AST/ALT ratio, also known as De Ritis ratio, is useful in assessing various liver diseases.[6] In

TABLE 3. DISEASE ASSOCIATION ACCORDING TO AMINOTRANSFERASES ELEVATION PATTERN

AST Predominant	ALT Predominant
Alcohol-related liver injury	Chronic hepatitis C
Cirrhosis	Chronic hepatitis B
Hemolysis	Acute viral hepatitis (types A-E, herpes simplex virus, Epstein-Barr virus, cytomegalovirus)
Myopathy	Steatosis/steatohepatitis
Thyroid disease	Hemochromatosis
Strenuous exercise	Medications/toxins
	Autoimmune hepatitis
	Wilson's disease
	Celiac disease

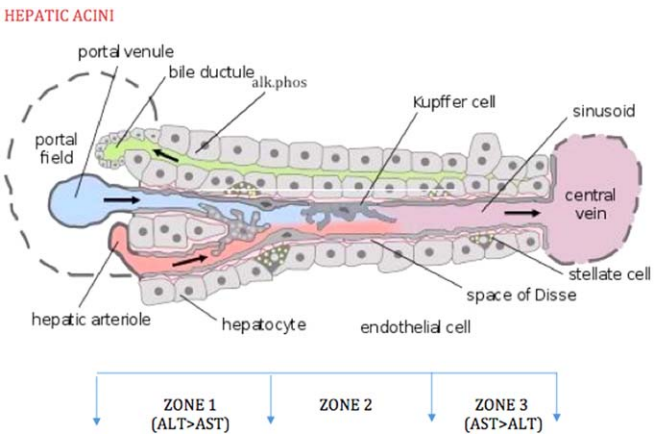

FIG 1 Zone 1 has more ALT than AST, and zone 3 has more AST than ALT. Autoimmune and viral hepatitis predominantly involve zone 1 (ALT > ALT). Ischemic and toxic events, heart failure, and Budd-Chiari syndrome predominantly involve zone 3 (AST > ALT). AP is mostly present on basolateral membrane of hepatocytes lining the bile canaliculi. Reproduced from *PLoS Biology*. Copyright 2005, Frevert et al.

alcoholic hepatitis, AST is usually higher than ALT, with the AST/ALT ratio reaching 2:1. In acute viral hepatitis, ALT levels are usually higher than AST. High AST/ALT ratio (>1.5) in acute viral hepatitis may be indicative of potential fulminant course.[9] AST/ALT ratio greater than 1.0 in chronic liver diseases may be indicative of advanced fibrosis.[8,9] AST and ALT are also used together with platelets to assess the likelihood of advanced liver fibrosis and are part of the aspartate aminotransferase-to-platelet ratio index (APRI) and FIB-4 score:

APRI: AST level (/ULN) / platelet counts (10^9/L) × 100

FIB-4 score: [age (years) × AST (U/L)]/{platelets (10^9/L) × [ALT (U/L)]$^{1/2}$}.

Aminotransferases are normal or only mildly elevated in obstructive jaundice except in acute phase of biliary obstruction caused by the passage of gallstone into the common bile duct.[10] In this case, aminotransferases may reach values greater than 1000, decreasing quickly, with liver test rapidly evolving into those of typical cholestasis or normalizing completely.

Aminotransferases levels also vary with age, sex, race, and body mass index.[11] Levels are found to be higher in obese patients and lower in dialysis patients,[12] whereas ALT levels are noted to decline with weight loss.[13] AST levels are 15% higher in African American males as compared with Caucasians.[11] Some individuals may have asymptomatic AST elevation caused by a defect in clearance of the enzyme.[14] Transaminases levels can be very

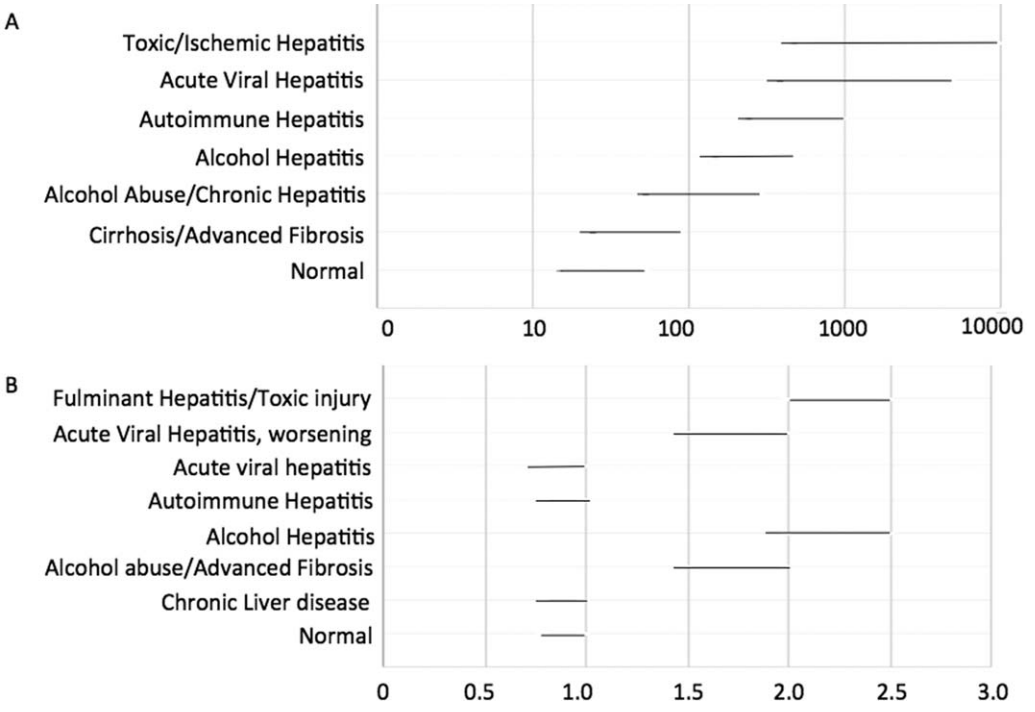

FIG 2 Typical AST elevation and De Ritis ratios for different kinds of liver diseases.

TABLE 4. ELEVATED AP

Hepatic	Nonhepatic
Bile duct obstruction	Bone disease
Benign intrahepatic recurrent cholestasis	Pregnancy
Primary biliary cholangitis	Chronic renal failure
Primary sclerosing cholangitis	Lymphoma and other malignancies
Medications	Congestive heart failure
Infiltrating diseases of the liver	Childhood growth
Sarcoidosis	
Hepatic metastasis	

high in patients with acute viral hepatitis, drug-induced liver injury, hepatic ischemia, and Budd-Chiari syndrome (Fig. 2). In asymptomatic patients with no underlying disease, mild aminotransferase elevation for more than 6 months warrants further investigation.[22]

MARKERS OF CHOLESTASIS

AP is the standard liver test reflecting cholestasis and can be complemented by gGT. gGT is part of a typical liver panel in some countries, whereas in the United States the standard liver test usually includes only AST, ALT, and AP. Because gGT is diffusely located in endoplasmic reticulum of bile ductal cells, its elevation is less specific for cholestasis but supports the suspicion that an elevated AP is liver derived as opposed to being of extrahepatic origin (Tables 1 and 4).[15]

Elderly individuals older than 60 years, especially women, may have a mildly elevated AP.[16] Individuals with blood types O and B may have an elevation of the serum AP after eating a fatty meal because of the influx of intestinal AP into circulation.[7] AP can also be nonpathologically elevated in children and adolescents undergoing rapid bone growth[16] and in women late in normal pregnancies because of the influx of placental AP.[17]

MARKERS OF LIVER FUNCTION

Bilirubin, albumin, and prothrombin time are standard tests to evaluate the liver function.

Bilirubin is the result of enzymatic breakdown of heme. Bilirubin is conjugated in the liver, resulting in water solubility. The conjugated bilirubin is then secreted into the bile. In healthy individuals, conjugated bilirubin comprises a small proportion of total bilirubin.[18]

In adults, unconjugated bilirubin elevation is most often of extrahepatic origin, mainly caused by hemolysis. In the absence of hemolysis, isolated unconjugated hyperbilirubinemia in an otherwise healthy patient should raise the suspicion for Gilbert syndrome. Up to 5% of the population has Gilbert syndrome, which is due to partial defects in uridine 5′-diphosphate-glucuronosyltransferase, the enzyme that conjugates bilirubin.[19] Crigler-Najjar syndrome is a rare cause of unconjugated hyperbilirubinemia.

In adults, conjugated hyperbilirubinemia is almost always a sign of biliary obstruction or impaired hepatic function. Two rare hereditary conditions cause defects in the secretory mechanism, Dubin-Johnson syndrome and Rotor syndrome, which result in elevated conjugated bilirubin.

Total serum bilirubin with increased prothrombin time correlates with poor outcomes in alcoholic hepatitis.[18] Both are also critical components of Model for End-Stage Liver Disease (MELD) score and Child-Pugh score.

Serum albumin is exclusively synthesized by hepatocytes, but the long half-life of albumin makes it difficult to interpret in the setting of acute liver injury. In chronic liver disease, albumin is the first of the three standard liver function tests to decline in advancing liver cirrhosis, before increase in bilirubin or prothrombin time. Albumin less than 35 g/dL should raise suspicion for cirrhosis. Differential diagnosis for hypoalbuminemia includes protein malnutrition of any cause, as well as protein-losing enteropathies, nephrotic syndrome, and chronic infection.

With the exception of factor VIII, all coagulation factors are synthesized in the liver. Because of the short half-lives of the coagulation factors, these are the best parameters to measure synthetic function of liver in acute conditions. This is most frequently done by prothrombin time determination. Because most clotting factors synthesized in the liver depend on vitamin K, prothrombin time is affected by vitamin K deficiency or use of vitamin K inhibitors. Vitamin K deficiency is seen in patients with chronic cholestasis or fat malabsorption from disease of the pancreas or small bowel. Prothrombin time is a better indicator of hepatic dysfunction than the international normalized ratio (INR),[20] despite INR

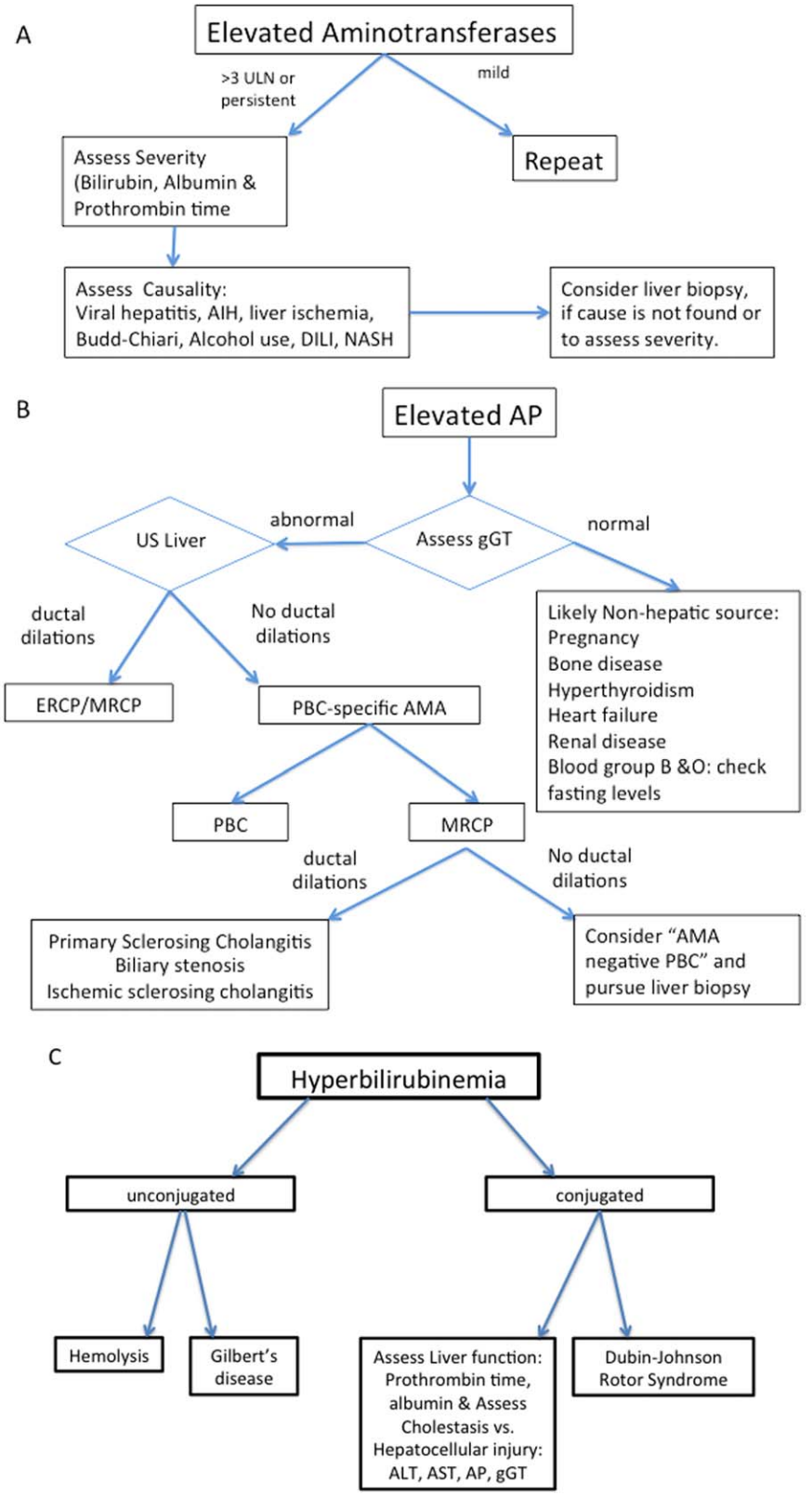

FIG 3 Algorithms for evaluation of elevated aminotransferases (A), AP (B), and bilirubin (C), respectively. Abbreviations: AIH, autoimmune hepatitis; AMA, antimitochondrial antibodies; DILI, drug-induced liver injury; ERCP, endoscopic retrograde cholangiopancreatography; MRCP, magnetic resonance cholangiopancreatography; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cholangitis.

having become a crucial part of the MELD score used for prioritizing liver allocations. In acute and chronic liver disease, prolonged prothrombin time (>5 seconds), which does not respond to parenteral vitamin K, is a poor prognostic sign.

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